PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :		(11) International Publication Number: WO 85/ 03639
A61K 39/04	A1	(43) International Publication Date: 29 August 1985 (29.08.85)

(21) International Application Number: PCT/GB85/00064

(22) International Filing Date: 15 February 1985 (15.02.85)

(31) Priority Application Number: 8404280

(32) Priority Date: 17 February 1984 (17.02.84)

(33) Priority Country: GB

(71) Applicant *(for all designated States except US):* UNIVERSITY COLLEGE LONDON [GB/GB]; Gower Street, London WC1E 6BT (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* STANFORD, John, Lawson [GB/GB]; Mill House, Claygate, Marden, Kent TN12 9PE (GB). ROOK, Graham, Arthur, William [GB/GB]; 27 Glenloch Road, London NW3 4DJ (GB).

(74) Agents: COLLIER, Jeremy, Austin, Grey et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5EU (GB).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BIOLOGICAL PREPARATIONS AND THEIR USE

(57) Abstract

Immunotherapeutic agents prepared from Mycobacterium vaccae are useful in the treatment of mycobacterial disease, especially tuberculosis or leprosy, in particular as an adjunct to chemotherapy.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BG	Bulgaria
BR	Brazil
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Germany, Federal Republic of
DK	Denmark
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

BIOLOGICAL PREPARATIONS AND THEIR USE

This invention relates to immunotherapeutic agents useful in the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy.

The eradication of mycobacterial diseases such as tuberculosis and leprosy by effective treatment is still a primary objective particularly in disease endemic areas such as third world countries of Asia, Africa and South East Asia. Modern drug treatment of these diseases consists of chemotherapy with, for example, rifampicin and isoniazid in the case of tuberculosis and clofazimine and sulphones in the case of leprosy.

Chemotherapy, though effective in killing rapidly metabolising bacilli, is very slow to eliminate "persisters", and this necessitates continuation of treatment for 9 months to a year in the case of tuberculosis, and 5 years or more in the case of leprosy. 'Persisters' are metabolically inactive microorganisms which can survive long exposure to a drug, only becoming susceptible when they start to multiply.

We have now found that the mycobacterium, M. vaccae, is especially effective for the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy. Experiments have shown that suspensions

containing killed M. vaccae in excess of 10^8 microorganisms per ml of diluent can be effective in eliminating "persisters" within a short period of time, usually 1 or 2 months. In addition, vaccines based on M. vaccae are easy to manufacture at low cost since M. vaccae can be cultivated in simple media, unlike some other species of mycobacteria, for example M. leprae, which can only be cultivated in armadillo tissues which are expensive and not easily obtainable.

The present invention therefore provides an immunotherapeutic agent comprising antigenic material derived from Mycobacterium vaccae. The antigenic material is conveniently, and therefore preferably, dead cells of M. vaccae, e.g. cells which have been killed by irradiation. The immunotherapeutic agent normally comprises more than 10^8 microorganisms per ml of diluent, and preferably from 10^8 to 10^{11} killed M. vaccae microorganisms per ml of diluent. The invention includes within its scope antigenic material from M. vaccae for use in therapy in the treatment of mycobacterial disease, e.g. tuberculosis or leprosy, preferably as an adjunct to chemotherapy.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent should be sterile. A suitable borate Buffer is:

$Na_2B_4O_7.10H_2O$	3.63 g

H_3BO_3	5.25 g
NaCl	6.19 g
Tween	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.C. Paul, Ann. Soc. belge Med, trop. 1973, 53, 141-389). The strain is a stable rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Jahasz., Zentr albl. Bakteriol. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133). M. vaccae is believed to be closely similar antigenically to M. leprae (J.L. Stanford et al, British Journal of Experimental Pathology, 1975, 56, 579).

The strain denoted R877R has been deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the immunotherapeutic agent, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955, 75, 15) solidified with agar.

Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated to enable growth of the microorganisms to take place, generally at 32°C for 10 days. The organisms are harvested, then weighed and suspended in a diluent. The diluent may be saline but it preferably also contains a surfactant such as Tween 80. 1 part Tween 80 is preferably used in 300 parts saline. The suspension is diluted with the saline/Tween 80 diluent to give 100 mg of microorganism/ml. For further dilution, borate buffered saline is preferably used so that the suspension contains 10 mg of microorganisms/ml of diluent. The suspension may then be dispensed into 5 ml multidose vials. The microorganisms in the vials are killed using irradiation e.g. from ^{60}Cobalt at a dose of 2.5 megarads, or by any other means, for example by heat.

The immunotherapeutic agent is in general administered by injection in a volume in the range 0.1-0.2 ml given intradermally. A single dosage may contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients suffering from mycobacterial disease a single dose containing 10^7 to 10^{10} killed M. vaccae. However, the dose may be repeated depending on the condition of the patient.

The immunotherapeutic agent is preferably

administered as an adjunct to chemotherapy, and normally 1 to 3 months after starting effective chemotherapy, e.g. with one of the chemotherapeutic agents mentioned above. Thus its effect is designed to be maximal after the majority of bacilli in the lesions, i.e. the metabolically active bacilli, have been killed and the load of bacterial antigenic material has begun to decline.

The invention therefore includes within its scope a method of treating mycobacterial disease, e.g. tuberculosis or leprosy, which comprises administering to a subject suffering therefrom antigenic material derived from Mycobacterium vaccae in an amount sufficient to provoke an immune response effective against metabolically inactive cells of mycobacteria.

The immunotherapeutic agent is believed to have two modes of action. It presents the "protective" common mycobacterial antigens to advantage and contains immune suppressor determinants active in regulating disadvantageous immune mechanisms (P.M. Nye et al, Leprosy Review, 1983, 54, 9). As a result of its action, "persister" bacilli are recognised by the immune system by their content of common mycobacterial antigens and effective immune mechanisms are directed against them, in the absence of the tissue necrotic form of immunity usually present in mycobacterial disease (G.A.W. Rook & J.L. Stanford, Parasite

Immunology, 1971, 1, 111). Thus "persisters" are eradicated by the action of the body defence mechanism and the period of chemotherapy required is drastically shortened. This dramatically reduces treatment costs, and the problem of patient non-compliance with chemotherapy.

It may be advantageous and is within the scope of the invention to use more than one strain of M. vaccae, and/or to include in the immunotherapeutic agent other mycobacterial antigens.

The immunotherapeutic agent may also contain BCG (Bacillus Calmette-Guerin) vaccine, in particular the freeze-dried form of the vaccine, to promote its effect.

The immunotherapeutic agent can contain further ingredients such as adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-dried form which is reconstituted prior to use.

The following Example illustrates the invention.

EXAMPLE

M. vaccae is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and incubated for 10 days at 32°C to enable growth of the microorganism to take place. The

microorganisms are then harvested and weighed and suspended in diluent (1 part Tween 80 in 300 parts saline) to give 100 mg of microorganisms/ml of diluent. The suspension is then further diluted with saline to give a suspension containing 10 mg of microorganisms/ml of diluent and dispensed into 5 ml multidose vials. The vials containing the live microorganism are then subjected to radiation from ^{60}Cobalt at a dose of 2.5 megarads to kill the microrganisms and give the immunotherapeutic agent of the invention, which may (if desired) be further diluted for use.

This immunotherapeutic agent may be administered by intradermal injection in the manner already described.

CLAIMS

1. An immunotherapeutic agent comprising antigenic material derived from Mycobacterium vaccae.

2. An immunotherapeutic agent according to claim 1 comprising dead cells of M. vaccae.

3. An immunotherapeutic agent according to claim 2 comprising cells of M. vaccae which have been killed by irradiation.

4. An immunotherapeutic agent according to any one of the preceding claims derived from M. vaccae NCTC 11659.

5. An immunotherapeutic agent according to any one of the preceding claims in the form of a single dosage unit containing 10^7 to 10^{10} killed cells of M. vaccae or antigenic material derived therefrom.

6. An immunotherapeutic agent according to any one of the preceding claims which also comprises BCG vaccine.

7. An immunotherapeutic agent according to any one of the preceding claims which also comprises one or more adjuvants, preservatives, and/or stabilisers.

8. An immunotherapeutic agent according to any one of the preceding claims in sterile injectable liquid form or in sterile freeze-dried form.

9. Antigenic material from Mycobacterium vaccae for use in therapy in the treatment of mycobacterial disease.

10. Dead cells of Mycobacterium vaccae for use in therapy in the treatment of mycobacterial disease.

11. Killed cells of Mycobacterium vaccae NCTC 11659 for use in therapy in the treatment of tuberculosis or leprosy.

12. An immunotherapeutic agent according to any one of claims 1 to 8 for use in therapy in the treatment of tuberculosis or leprosy.

13. Antigenic material from Mycobacterium vaccae for use in therapy in the treatment of mycobacterial disease as an adjunct to chemotherapy.

14. Method of treating mycobacterial disease which comprises administering to a subject suffering therefrom antigenic material derived from Mycobacterium vaccae in an amount sufficient to provoke an immune response effective against metabolically inactive cells of mycobacteria.

15. Method according to claim 14 in which the mycobacterial disease is tuberculosis or leprosy and the mycobacteria are Mycobacterium tuberculosis or M. leprae.

16. Method according to claim 14 in which the antigen material comprises dead cells of M. vaccae.

17. Method according to claim 14 in which the antigenic material comprises cells of M. vaccae NCTC11659 which have been killed by irradiation.

18. Method according to claim 14 in which

chemotherapy is also used to kill metabolically active cells of mycobacteria.

19. Method according to claim 18 in which the metabolically active cells are first killed by chemotherapy and the antigenic material from M. vaccae is then administered to provoke an immune response against the metabolically inactive cells.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00064

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 39/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Infection and Immunity, volume 20, no. 2, May 1978, Washington (US) F.M. Collins et al.: "Immune response to persistent Mycobacterial infection in mice", see pages 430-438, especially page 437, lines 30-54 --	1-13
X	Biological Abstracts, volume 69, no. 1, 1980, Philadelpha (US) S.R. Watson et al.: "Delayed hypersensitivity responses in mice and guinea pigs to Mycobacterium leprae, Mycobacterium vaccae and Mycobacterium nonchromogeni cum cytoplasmic proteins", see page 306, abstract 2847, Infect.Immun. 25(1)229-236,1979 --	1-13
X	Biological Abstacts, volume 78, no. 3, 1984, Philadelphia (US) F.M. Collins et al.: "Fernandez and Mitsuda reactivity in guinea pigs sensitized with heat-killed Mycobacterium leprae: persistence and	

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
9th May 1985	21 JUIN 1985
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	specificity of skin reactivity to soluble and particulate antigens", see page 2213, abstract 19506, Int.J. Lepr. 51(4): 481-489, 1983	1-13

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 14-19 because they relate to subject matter not required to be searched by this Authority, namely:

Method for treatment of the human or animal body by therapy;

PCT Rule 39.1.(iv)

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.